

Claims

1. A polynucleotide, characterized in that it comprises a marker gene which is inactivated by the 5 insertion of an *Impala* transposon, said marker gene comprising, in the direction of transcription, a promoter regulatory sequence which is functional in *Magnaporthe grisea* and which is functionally linked to the coding sequence of said marker gene.

10 2. The polynucleotide as claimed in claim 1, characterized in that the promoter regulatory sequence is a promoter regulatory sequence of a gene from *Magnaporthe grisea*, or from another fungus, in particular from a filamentous fungus.

15 3. The polynucleotide as claimed in either of claims 1 and 2, characterized in that the promoter regulatory sequence consists of the promoter regulatory sequence of a fungal *niaD* or *gpdA* gene.

20 4. The polynucleotide as claimed in claim 3, characterized in that the promoter regulatory sequence is a promoter regulatory sequence of the *niaD* gene from *Aspergillus nidulans*, which is functional in *Magnaporthe grisea*.

25 5. The polynucleotide as claimed in claim 4, characterized in that the promoter regulatory sequence of the *niaD* gene from *Aspergillus nidulans* is more than 0.4 kb long.

6. The polynucleotide as claimed in one of claims 1 to 5, characterized in that the coding sequence of a marker gene is chosen from the coding sequences of a reporter gene, in particular GUS or GFP,
5 the coding sequences for a gene for tolerance to an antibiotic or herbicide, in particular the genes for resistance to hygromycin (*hph*), to phleomycin (*ble*) or to the herbicide bialaphos (*Bar*), or a gene for resistance to sulfonylureas.

10 7. The polynucleotide as claimed in one of claims 1 to 5, characterized in that the marker gene is chosen from the genes encoding enzymes which are functional in fungi, in particular encoding a nitrate reductase (*niaD*) or a nitrilase.

15 8. The polynucleotide as claimed in claim 7, characterized in that the marker gene is the nitrate reductase gene from *Aspergillus nidulans*.

9. The polynucleotide as claimed in one of claims 1 to 8, characterized in that the *Impala* 20 transposon is integrated into the promoter regulatory sequence of the polynucleotide as claimed in the invention.

10. The polynucleotide as claimed in one of claims 1 to 9, characterized in that the *Impala* 25 transposon comprises a marker gene.

11. The polynucleotide as claimed in one of claims 1 to 10, characterized in that the *Impala*

transposon is defective.

12. A method for preparing insertion mutants of fungi, characterized in that it comprises the following steps:

- 5 a) said fungus is transformed with a polynucleotide comprising a marker gene which has been inactivated by the insertion of an *Impala* transposon as claimed in one of claims 1 to 10, under conditions which allow the excision of the *Impala* transposon of said marker gene and its reinsertion into the genome of the fungus;
- 10 b) the insertion mutants expressing the marker gene are identified.

13. A method for preparing insertion mutants of fungi, characterized in that it comprises the following steps:

- a) said fungus is transformed with a polynucleotide comprising a marker gene which has been inactivated by the insertion of a defective *Impala* transposon as claimed in claim 11;
- 20 b) the defective *Impala* transposon is mobilized using a transposase, the expression of which is controlled, under conditions which allow the excision of the defective *Impala* transposon, its reinsertion and its stabilization in the genome of the fungus;
- 25 c) the insertion mutants expressing the marker gene

are identified.

14. A method for identifying a gene associated with a detectable phenotype in fungi, characterized in that it comprises the following steps:

- 5 a) insertion mutants are prepared by inserting an *Impala* transposon into the genome of said fungi according to one of the methods of claims 12 or 13;
- b) at least one insertion mutant with said detectable phenotype is selected;
- 10 c) the gene into which, or close to which, the *Impala* transposon has inserted is isolated.

15. A host organism transformed with a polynucleotide as claimed in one of claims 1 to 11.

16. The host organism as claimed in claim 15, characterized in that the host organism is a fungus.

17. A fungus into the genome of which is integrated a polynucleotide as claimed in one of claims 20 1 to 11.

18. The fungus as claimed in claim 17, characterized in that the marker gene is a fungal nitrate reductase gene and the fungus is nia-.

19. An insertion mutant of filamentous fungi chosen from the fungi of the *Magnaporthe* or *Penicillium* genera, into the genome of which is integrated the *Impala* transposon.